Effects of Weight Loss and Exercise on the Distribution of Lead and Essential Trace Elements in Rats with Prior Lead Exposure

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We studied the effects of weight loss and non-weight-bearing exercise (swimming) on blood and organ lead and essential metal concentrations in rats with prior lead exposure. Nine-week-old female Sprague-Dawley rats (n = 37) received lead acetate in their drinking water for 2 weeks, followed by a 4-day latency period without lead exposure. Rats were then randomly assigned to one of six treatment groups: weight maintenance with *ad libitum* feeding, moderate weight loss with 20% food restriction, and substantial weight loss with 40% food restriction, either with or without swimming. Blood lead concentrations were measured weekly. The rats were euthanized after a 4-week period of food restriction, and the brain, liver, kidneys, quadriceps muscle, lumbar spinal column bones, and femur were harvested for analysis for lead, calcium, copper, iron, magnesium, and zinc using atomic absorption spectrophotometry. Both swimming and nonswimming rats fed restricted diets had consistently higher blood lead concentrations than the *ad libitum* controls. Rats in the substantial weight loss group had higher organ lead concentrations than rats in the weight maintenance group. Rats in the moderate weight loss group had intermediate values. There were no significant differences in blood and organ lead concentrations between the swimming and nonswimming groups. Organ iron concentrations increased with weight loss, but those of the other metals studied did not. Weight loss also increased hematocrits and decreased bone density of the nonswimming rats. The response of lead stores to weight loss was similar to that of iron stores because both were conserved during food restriction in contrast to decreased stores of the other metals studied. It is possible that weight loss, especially rapid weight loss, could result in lead toxicity in people with a history of prior excessive lead exposure. *Key words*: exercise, food restriction, iron, lead, rat, swimming, weight loss. *Environ Health Perspect* 107:657–662 (1999). [Online 30 June 1999]
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Many people lose weight, either intentionally or due to illness. Intentional weight loss can be accomplished by reducing food intake (food restriction) and/or increasing energy expenditure (*1,2*). When body mass is lost, there is a decrease in water, fat, and lean mass, including bone mass (*3–7*). It is well established that reducing body weight can decrease the risk for several chronic diseases in people who are overweight (*8–11*). However, there is some evidence that body mass loss that is too rapid or excessive may be harmful to health (*12–14*) and may even increase mortality in people ≥ 50 years of age (*15*). The hypothesis that the mobilization of stored environmental toxicants during weight loss may contribute to these effects has not been previously addressed.

Millions of men and women have been exposed to excessive amounts of lead since childhood. Exposure at an early age results in greater Pb retention (*16*). Because more than 90% of the body Pb burden is stored in bone and has a very long half-life (*17*), up to 20 years or more in cortical bone, most adults have substantial skeletal Pb stores that are about 500-fold greater than those of our preindustrial ancestors (*18*).

Because weight loss induced by food restriction may be accompanied by loss of bone mass (*3–7*), reduced skeletal mass during food restriction can result in the release of minerals and trace elements from bone into the bloodstream. Release of bone Pb may exert adverse effects on cells and organs sensitive to Pb toxicity, especially when bone-mass loss is rapid. However, exercise, by modifying the loss of bone mass during energy restriction, may influence the mobilization of Pb from bones to soft tissues. The type of exercise may be a factor because the effects of weight-bearing exercise may differ from those of non-weight-bearing exercise.

A previous study demonstrated that weight loss could increase the quantity and concentration of Pb in the liver and kidney, even in the absence of continued Pb exposure (*19*). However, no prior studies have monitored blood Pb concentrations during food restriction, and it is not known if either weight-bearing or non-weight-bearing exercise can influence blood and tissue Pb concentrations and contents during or after the period of food restriction. The objective of this study was to determine the effects of weight loss induced by food restriction, with and without non-weight-bearing exercise (swimming), on organ weights, bone density, hematocrit, and blood and organ Pb concentrations in rats exposed to Pb prior to weight loss. A second goal was to contrast effects on Pb with changes in concentrations of five essential metals: calcium, copper, iron, magnesium, and zinc. A third goal was to obtain data to support hypotheses for studies of the mechanisms by which weight loss can influence Pb toxicity.

Methods

Animal care. Thirty-seven 9-week-old female Sprague-Dawley rats were individually housed in plastic cages in a standard animal facility environment. A 12-hr light/dark cycle was maintained along with a room temperature of 22°C. After 1 week of acclimatization, all rats were given 250 µg/mL Pb as the acetate in their drinking water. Glacial acetic acid was added to the water at a concentration of 12.5 µL/L to prevent the precipitation of Pb carbonate and hydroxides. The Pb-containing water consumed by the rats was monitored and changed weekly. After 14 days of Pb exposure, the rats were then given distilled drinking water. Following a 4-day latency period without exposure to Pb, they were randomly assigned to one of six treatment groups: weight maintenance (WM) fed *ad libitum*, moderate weight loss (MWL), and substantial weight loss (SWL), either with or without swimming. There were six rats in each group except the swimming SWL group, which had seven rats. The rats in the WM group had free access to the diet, but the rats in the MWL and SWL groups were restricted to 80% and 60%, respectively, of the diet consumed by the WM group. Food consumption was monitored daily during the 4-week period of food restriction. We used the Pure Laboratory Rodent Diet, 5001 (PMI Feeds, Inc., St. Louis, MO). The vitamin D_3 content of this diet was 4.5 IU/g. The manufacturer's values for the mineral and trace element content of the diet for Ca, Cu, Fe, Mg, and Zn were 1.00%, 18.0 µg/g, 198 µg/g, 0.21%, and 70.0 µg/g, respectively. The diet was analyzed by atomic absorption

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spectrophotometry as described under "Laboratory Analyses." Our laboratory results for these elements were 1.02% (Ca), 13.5 µg/g (Cu), 200 µg/g (Fe), 0.20% (Mg), and 81.9 µg/g (Zn), based on analysis in triplicate. The Pb concentration in the diet was 0.25 µg/g. Rat body weight was determined once each week before food restriction and twice each week during the food restriction period using a mechanical triple beam balance (Ohaus, Florham Park, NJ).

Swimming. Rats swam in plastic containers (0.70 m × 0.45 m × 0.40 m deep). The water temperature was maintained at 36 ± 1°C (*20,21*). All rats were trained to swim for 15 min/day for 5 days before the start of food restriction. On the first day of food restriction, only the rats in the swimming groups continued to swim for 15 min, and their swimming time was gradually increased by 15 min every 1–2 days until a maximum of 120 min/day was attained. The swimming time remained at 120 min/day for the last 2 weeks of food restriction.

Sample collection. We collected tail vein blood samples once each week before and during food restriction. The rat tail was soaked in warm water (45°C) for 1 min and then dried with a paper towel. After the venous blood return was restricted and pooled with gentle finger pressure at the tail root, a heparin pre-rinsed 26-gauge needle was inserted into a tail vein near the end of the tail. A gentle caudal stroke was applied by a second investigator to cause blood to flow to the needle. A blood sample of about 150 µL was transferred with heparinized capillary tubes (two tubes) to a heparinized microcentrifuge tube, and then stored at -70°C for future analysis for Pb. One extra capillary tube of blood was collected and centrifuged for determination of the hematocrit.

At the end of the 4-week period of food restriction, blood was drawn by cardiac puncture from rats anesthetized with 25 mg/kg sodium pentobarbital (Nembutal sodium solution, 50 mg/mL; Abbott Laboratories, North Chicago, IL). Rats were then decapitated while under heavy anesthesia. The organs harvested from each rat were the liver, kidneys, left femur, brain, quadriceps muscle, and bones of the lower vertebral column. Organs were briefly immersed in deionized distilled water to remove surface blood contamination. After air drying, organs were stored in pre-rinsed polyethylene or polypropylene containers at -20°C.

A protocol describing the above procedures was approved by the Institutional Animal Care and Use Committee of the New Jersey Medical School.

Laboratory analyses. Whole-blood Pb concentrations were determined by electrothermal atomic absorption spectrophotometry. A quality control sample, Bio Rad Whole Blood Control Level 3 (Bio-Rad, Anaheim, CA), was used for evaluation of the accuracy of these analyses. Concentrations determined for this sample were within 8% of the certified value. Rat feed and organ concentrations of Ca, Cu, Fe, Mg, and Zn were determined by previously described techniques (*16*). Briefly, organ or feed samples were digested with a 3:1 mixture of double distilled nitric and perchloric acids (GFS Chemicals, Columbus, OH), and the residue was quantitatively transferred to a 10- or 25-mL volumetric flask and diluted with deionized distilled water. Further dilutions were necessary for some analyses, which were conducted by using flame atomic absorption spectrophotometry (Perkin-Elmer Model 603; Perkin-Elmer, Norwalk, CT). Bovine liver from the National Institutes of Standards and Technology (NIST 1577b, Gaithersburg, MD) was used as a quality control sample for all analyses for Ca, Cu, Fe, Mg, and Zn. Assays of the bovine liver sample in our laboratory gave results within 5% of the certified values. Lead concentrations of the same samples were determined by electrothermal atomic absorption spectrophotometry (Perkin-Elmer Model 503 with HGA-2200 Heated Graphite Atomizer; Perkin-Elmer, Norwalk, CT). Orchard leaves (NIST 1571) was used as a quality control sample for analysis for tissue Pb because the Pb concentration of NIST bovine liver is too low to be useful for quality control for this metal. The precision coefficient of variation (CV) of the assays, based on analysis of the standard reference materials and/or quality control samples, was 0.01–0.07. Calculations of concentrations were based on wet tissue weight.

Specific gravity (density) of rat femurs was determined by measuring the air weight (W_a) and water-submerged weight (W_{ws}) of the specimens using an electromechanical balance (Sartorius, Edgewood, NY). The femurs were first individually soaked in deionized distilled water at room temperature for 24 hr; the specimens were then air dried for 10 min on glassine papers. After determining the W_a by hanging the femur head in a loop of string on the balance, we measured the W_{ws} by submerging a suspended femur completely in deionized distilled water. We subtracted the string weight (W_s) from W_a and W_{ws} when calculating the density, which we determined using the following formula (*22*):

$$\text{Density} = \frac{(W_a - W_s)}{(W_a - W_s) - (W_{ws} - W_s)}$$

Statistics. Dietary restriction will necessarily result in loss of mass of some organs. Therefore, in addition to organ metal concentrations, we also calculated the total organ metal contents (concentration × organ weight). Values shown in the text are mean ± standard error (SE). Data reduction and analysis were performed using dBASE III+ (Ashton-Tate, Torrance, CA) and the Statistical Analysis System (SAS Institute, Cary, NC). We used analysis of variance (ANOVA) to evaluate the effects of food restriction and non-weight-bearing exercise on blood and organ metal concentrations and contents. If the ANOVA indicated that there were statistically significant differences ($p < 0.05$) among the six treatment groups for a specific measurement, pair-wise comparisons were made by Duncan's multiple range test at $\alpha = 0.05$. The interactions of the two treatment factors of food restriction and swimming were analyzed using two-way ANOVA. We evaluated the concentrations of blood Pb and the concentrations and organ contents of Pb and the essential metals both for the six treatment groups and for the three food restriction groups after combining the data of the swimming and nonswimming rats.

Results

Water consumption and body and organ weights. The daily drinking water consumption of the rats during the 14-day period of Pb exposure was 31.0 ± 1.3 mL (mean ± SE). At a concentration of 250 µg/mL, the mean daily intake of Pb from water was 7,750 µg. The daily consumption of Pb-containing drinking water and rat body weights at the beginning of food restriction were comparable in the six groups (ANOVA, $p > 0.05$). The daily food intake of nonswimming WM rats was 15 ± 1 g (mean ± SE). At a concentration of 0.25 µg/g, the mean daily intake of Pb from food was only 3.8 µg. The rats in the swimming groups consumed approximately 3 g more food per day than those in the nonswimming groups. At the end of the 4-week period of food restriction, the rats in the MWL group had lost 6% of their body weight as compared to their body weight before food restriction; the rats in the SWL group lost 18%, and the rats in the WM group had a 6% weight gain (Figure 1). The body weights of the swimming groups and nonswimming groups were not significantly different (ANOVA, $p > 0.05$).

Liver and kidney weights of the food restriction groups (MWL, SWL) were lower than those of both the swimming and nonswimming WM groups (Table 1). The trends were WM > MWL > SWL. Weights of other organs did not differ significantly among the treatment groups. There were no significant differences in organ weights between swimming and nonswimming rats. For nonswimming rats but not swimming rats, femur density was significantly lower for

rats in the food restriction groups than for rats in the WM group ($p < 0.05$) (Figure 2).

Lead concentrations and organ content. The blood Pb concentration of all rats before food restriction was 1.65 ± 0.05 µmol/L (mean ± SE), and there were no significant differences among the six groups (ANOVA, $p > 0.05$). The blood Pb concentrations decreased gradually during the food restriction period (Figures 3 and 4). Figure 4 is enlarged from Figure 3 (days 27 and 33) to better illustrate differences between treatment groups. The rats in the SWL groups had the highest blood Pb concentrations and the WM groups had the lowest blood Pb concentrations for both the swimming and nonswimming rats. Food restriction was initiated on day 18 of the study. The ratios of blood Pb concentrations of the SWL groups to those of WM groups on study days 7, 13, 20, 27, 33, 40, and 47 for nonswimming rats were 0.93, 1.01, 1.37, 1.71, 1.59, 1.85, and 1.60, respectively. For the swimming rats, ratios were 0.98, 1.18, 1.20, 1.17, 1.21, 1.18, and 1.14, respectively, and for combined groups of swimming and nonswimming rats, ratios were 0.96, 1.10, 1.28, 1.46, 1.38, 1.48, and 1.34, respectively. When the data for nonswimming and swimming groups were combined, the blood Pb concentrations of the SWL group were significantly higher than those of the WM group (ANOVA, $p < 0.05$) for study days 20–47. However, there were no significant differences in blood Pb concentrations between the swimming and nonswimming groups, even though the nonswimming rats had consistently higher blood Pb concentrations during the period of food restriction. The hematocrits of the SWL group were higher than those of the WM group (Figure 5) for days 27, 33, and 40. There was a significant positive correlation between the blood Pb concentrations and the hematocrit. The correlation coefficients were 0.36 ($p = 0.036$), 0.47 ($p = 0.004$), and 0.31 ($p = 0.067$) for blood drawn on days 27, 33, and 40, respectively.

Liver Pb concentrations differed significantly among the six treatment groups; rats

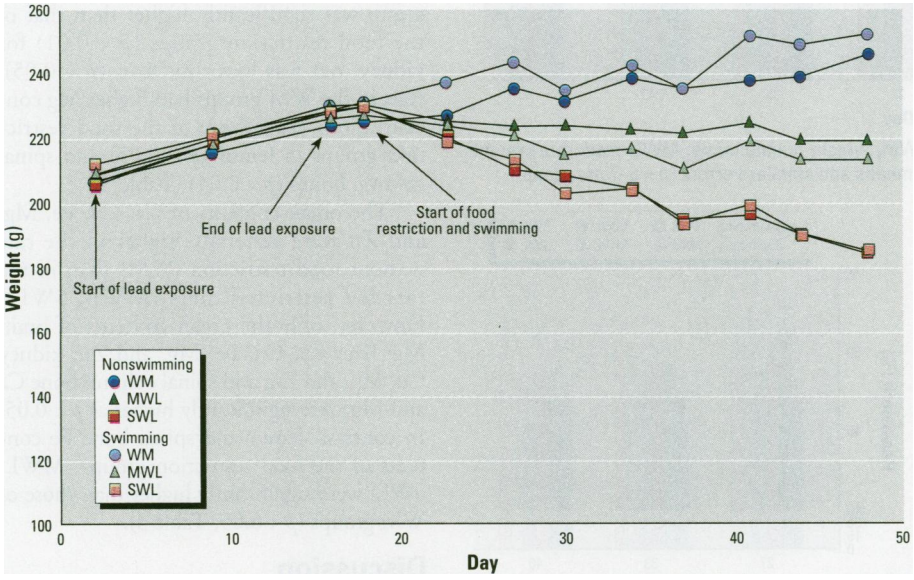

Figure 1. Growth curves (body weight) for rats that ingested 250 µg/mL lead in their drinking water for 14 days beginning at 9 weeks of age. Abbreviations: WM, weight maintenance; MWL, moderate weight loss; SWL, substantial weight loss.

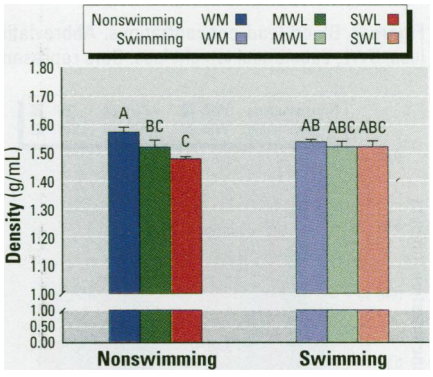

Figure 2. Femur density. Bars represent the means ± standard errors (n = 5–7 per group). Abbreviations: WM, weight maintenance; MWL, moderate weight loss; SWL, substantial weight loss. Concentrations not marked with the same letter differ significantly (Duncan's test, $p < 0.05$).

Table 1. Tissue lead concentrations and contents.

Organ	Group	Organ weight (g)			Lead concentration (nmol/g)			Lead content (nmol)		
		None	Swim	Combined	None	Swim	Combined	None	Swim	Combined
Brain	WM	1.91 ± 0.04	1.93 ± 0.05	1.92 ± 0.03	0.56 ± 0.06	0.74 ± 0.14	0.64 ± 0.07	0.98 ± 0.08	1.39 ± 0.25	1.18 ± 0.14
	MWL	1.88 ± 0.03	1.88 ± 0.02	1.88 ± 0.02	0.58 ± 0.07	0.58 ± 0.07	0.58 ± 0.05	1.10 ± 0.14	1.09 ± 0.13	1.09 ± 0.09
	SWL	1.80 ± 0.09	1.86 ± 0.03	1.83 ± 0.04	0.76 ± 0.06	0.69 ± 0.13	0.72 ± 0.07	1.35 ± 0.08	1.29 ± 0.24	1.32 ± 0.13
Femur	WM	0.62 ± 0.02	0.63 ± 0.01	0.63 ± 0.01	116 ± 9	134 ± 6	124 ± 6	64.7 ± 4.0	86.8 ± 5.3	75.7 ± 4.6
	MWL	0.65 ± 0.01	0.60 ± 0.01	0.62 ± 0.01	142 ± 16	124 ± 8	133 ± 9	84.2 ± 7.7	76.2 ± 5.5	80.2 ± 4.6
	SWL	0.60 ± 0.01	0.61 ± 0.02	0.61 ± 0.01	149 ± 7	144 ± 16	147 ± 9	94.8 ± 4.4	88.7 ± 11.3	90.9 ± 6.7
Kidney	WM	0.81 ± 0.02A	0.78 ± 0.03A	0.79 ± 0.02a	2.92 ± 0.29	3.71 ± 0.67	3.28 ± 0.33b	2.39 ± 0.27	2.99 ± 0.59	2.66 ± 0.31
	MWL	0.72 ± 0.04B	0.70 ± 0.02B	0.71 ± 0.02b	4.47 ± 0.81	3.90 ± 0.42	4.18 ± 0.44ab	3.12 ± 0.49	2.72 ± 0.28	2.92 ± 0.28
	SWL	0.61 ± 0.02C	0.59 ± 0.02C	0.60 ± 0.01c	5.39 ± 0.76	4.60 ± 0.84	4.96 ± 0.56a	3.25 ± 0.43	2.80 ± 0.61	3.01 ± 0.37
Liver	WM	9.20 ± 0.79A	9.57 ± 0.42A	9.38 ± 0.43a	0.34 ± 0.10B	0.30 ± 0.14B	0.33 ± 0.05b	3.15 ± 0.93	2.99 ± 0.97	3.09 ± 0.62
	MWL	6.63 ± 0.30BC	6.86 ± 0.17B	6.75 ± 0.17b	0.23 ± 0.07B	0.68 ± 0.17AB	0.46 ± 0.11ab	1.55 ± 0.46	4.78 ± 1.22	3.17 ± 0.79
	SWL	5.80 ± 0.23BC	5.45 ± 0.18C	5.61 ± 0.15c	0.65 ± 0.15AB	0.86 ± 0.18A	0.76 ± 0.12a	3.62 ± 0.74	4.56 ± 0.90	4.13 ± 0.59
Muscle	WM	1.42 ± 0.11	1.67 ± 0.11	1.54 ± 0.08	0.26 ± 0.09	0.14 ± 0.03	0.20 ± 0.05			
	MWL	1.47 ± 0.07	1.46 ± 0.08	1.46 ± 0.05	0.20 ± 0.04	0.16 ± 0.02	0.18 ± 0.02			
	SWL	1.26 ± 0.14	1.48 ± 0.03	1.38 ± 0.07	0.24 ± 0.05	0.27 ± 0.07	0.25 ± 0.04			
Spinal column bone	WM	0.91 ± 0.04	0.98 ± 0.04	0.95 ± 0.03	118 ± 7	131 ± 8	124 ± 5	105 ± 8	131 ± 8	120 ± 7
	MWL	0.91 ± 0.04	0.96 ± 0.03	0.94 ± 0.03	150 ± 13	128 ± 13	139 ± 9	140 ± 18	124 ± 15	132 ± 11
	SWL	0.82 ± 0.02	0.95 ± 0.04	0.88 ± 0.03	153 ± 12	157 ± 17	155 ± 10	126 ± 12	152 ± 24	138 ± 13

Abbreviations: WM, weight maintenance (diet not restricted); MWL, moderate weight loss (diet restricted to 80% of WM group diet); SWL, substantial weight loss (diet restricted to 60% of WM group diet); None, nonswimming rats (n = 5–6); Swim, swimming rats (n = 5–7); Combined, both nonswimming and swimming rats (n = 10–13). Data are means ± standard errors. Values for each organ not marked with the same capital or lower case letter differ significantly (Duncan's test, $p < 0.05$). Capital letters are used for nonswimming or swimming groups and lower case letters identify significant differences among combined groups.

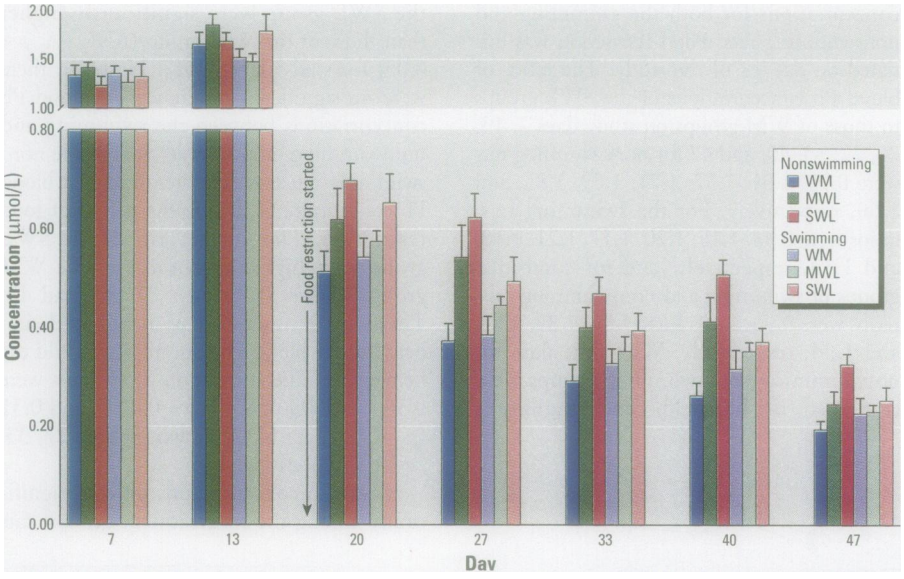

Figure 3. Blood lead concentrations. Abbreviations: WM, weight maintenance; MWL, moderate weight loss; SWL, substantial weight loss. Bars represent the means and standard errors (n = 5–7 per group).

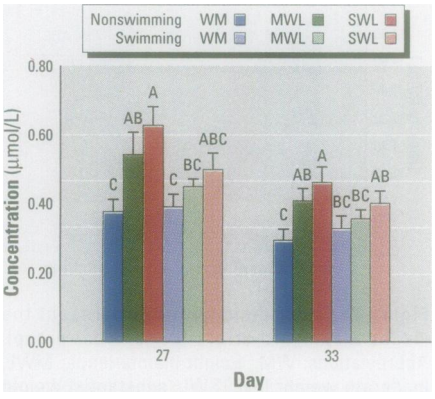

Figure 4. Blood lead concentrations for study days 27 and 33. Abbreviations: WM, weight maintenance; MWL, moderate weight loss; SWL, substantial weight loss. Bars represent the means and standard errors (n = 5–7 per group). Concentrations not marked with the same letter differ significantly (Duncan's test, $p < 0.05$).

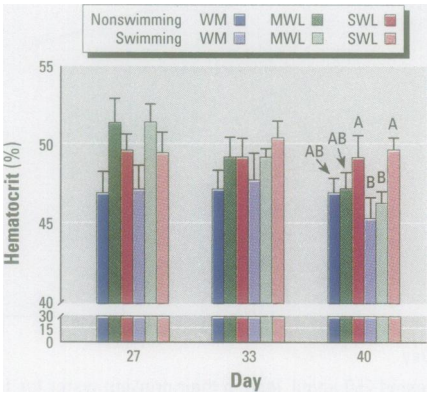

Figure 5. Rat hematocrits for study days 27, 33, and 40. Abbreviations: WM, weight maintenance; MWL, moderate weight loss; SWL, substantial weight loss. Bars represent the means and standard errors (n = 5–7 per group). Concentrations not marked with the same letter differ significantly (Duncan's test, $p < 0.05$).

in the food restriction groups (MWL and SWL) had higher Pb concentrations than rats in the weight maintenance groups (WM) for both swimming and nonswimming rats (Table 1). Analysis of kidney Pb concentrations of the six treatment groups by Duncan's multiple range test showed that the kidney Pb of nonswimming SWL rats was higher than that of nonswimming WM rats, but the ANOVA did not confirm statistical significance at p = 0.05. When compared within the three groups of nonswimming rats by ANOVA, the kidney Pb concentration of SWL rats was significantly higher than that of WM rats ($p < 0.05$). The Pb concentrations of brain, femur, muscle, and spinal column bone did not differ significantly among the six treatment groups, even though the Pb concentrations of the SWL groups were generally the highest in these tissues.

There were no significant differences in Pb concentrations between nonswimming and swimming rats. When the data for nonswimming and swimming groups were combined, organ Pb concentrations followed the order SWL > MWL > WM for all organs or tissues analyzed except brain and skeletal muscle, but only the liver and kidney Pb concentrations were significantly different among the three food restriction groups. The organ Pb contents were not significantly different for all organs and tissues even though the organ weights of liver and kidney of the food restriction groups were significantly lower than those of the WM groups (Table 1).

Essential metal concentrations and organ content. The organ Fe concentrations of the food restriction groups (MWL and SWL) were significantly higher than those of the WM groups for all organs and tissues except brain and were not influenced by swimming. The combined organ Fe concentrations of the swimming and nonswimming rats followed the order SWL > MWL > WM (ANOVA, $p < 0.05$; Table 2).

Organ Ca concentrations did not differ significantly except for the femur Ca concentration, which was slightly but significantly higher in the WM group ($p < 0.05$). The liver Zn concentration of the WM group was slightly but significantly lower than that of the food restriction groups ($p < 0.01$). The Cu concentration of the WM group was significantly higher than that of the food restriction groups ($p < 0.01$) for kidney, but was lower for liver ($p < 0.05$). Rats in the WM groups had higher Mg concentrations than those of the food restriction groups in femur (p = 0.03) and spinal column bones (p = 0.04) (Table 2).

The organ contents of Ca, Cu, Fe, Mg, and Zn were generally higher in the rats without food restriction (WM) than in the rats fed restricted diets (MWL, SWL). However, only the organ contents of brain Mg; liver Ca, Cu, Fe, Mg, and Zn; kidney Cu, Mg, and Zn; and spinal column bone Ca and Mg were significantly higher at $p < 0.05$. In contrast, femur and spinal bone Fe contents of the food restriction groups (MWL, SWL) were significantly higher than those of WM groups ($p < 0.05$; Table 3).

Discussion

The benefits of weight loss for overweight people, especially those with a body mass index > 30 kg/m^2, have been widely documented. These benefits include decreasing the risks of hypertension, cardiovascular disease, some cancers, adult-onset diabetes mellitus, and strokes (*8–11*). However, substantial weight loss, especially if rapid, can result in immune dysfunction (*12–14,23,24*) and hormonal and metabolic changes (*25*). Food restriction will inevitably result in decreased intake of essential minerals and micronutrients unless accompanied by the use of micronutrient supplements. The present study best approximates food restriction in people who do not consume micronutrient supplements while losing weight. Thus, the results are particularly applicable to those people who do not have access to micronutrient supplements or cannot afford to purchase them; this includes the majority of people in the world.

Among the organs harvested in this study, the weights of the liver and kidney were reduced the most by food restriction. Even though the femur and spinal column bone weights did not differ significantly among groups after 4 weeks of food restriction, the

Table 2. Organ concentrations of essential metals.

Organ	Group	Calcium (μmol/g)	Copper (nmol/g)	Iron (μmol/g)	Magnesium (μmol/g)	Zinc (μmol/g)
Brain	WM	1.63 ± 0.31	41.6 ± 0.8	0.312 ± 0.010	6.34 ± 0.08	0.189 ± 0.001
	MWL	1.79 ± 0.50	40.1 ± 0.6	0.329 ± 0.008	6.22 ± 0.04	0.192 ± 0.001
	SWL	1.33 ± 0.17	41.3 ± 0.8	0.323 ± 0.005	6.10 ± 0.08	0.188 ± 0.001
Femur	WM	5,156 ± 49 A	53.9 ± 1.4	0.690 ± 0.033 B	165 ± 2 A	2.87 ± 0.04
	MWL	4,959 ± 55 B	66.7 ± 14.2	0.870 ± 0.052 A	156 ± 3 B	2.75 ± 0.05
	SWL	5,009 ± 60 AB	55.4 ± 1.1	0.995 ± 0.073 A	158 ± 2 B	2.78 ± 0.06
		$p = 0.046$		$p = 0.002$	$p = 0.033$	
Kidney	WM	8.17 ± 2.76	245 ± 16 A	1.72 ± 0.057 B	8.73 ± 0.23	0.358 ± 0.008
	MWL	13.72 ± 5.17	159 ± 5 B	1.86 ± 0.067 B	8.74 ± 0.28	0.366 ± 0.004
	SWL	12.07 ± 3.48	190 ± 13 B	2.09 ± 0.075 A	8.36 ± 0.21	0.375 ± 0.008
			$p = 0.001$	$p = 0.002$		
Liver	WM	0.65 ± 0.02	65.2 ± 2.4 B	5.32 ± 0.22 C	8.14 ± 0.16	0.383 ± 0.009 B
	MWL	0.67 ± 0.02	72.2 ± 1.5 AB	6.46 ± 0.21 B	8.66 ± 0.14	0.449 ± 0.014 A
	SWL	0.63 ± 0.02	73.9 ± 3.2 A	7.59 ± 0.27 A	8.41 ± 0.17	0.429 ± 0.017 A
			$p = 0.047$	$p = 0.001$		$p = 0.007$
Muscle	WM	1.22 ± 0.05	15.6 ± 0.7	0.260 ± 0.008 B	10.1 ± 0.5	0.212 ± 0.015
	MWL	1.12 ± 0.07	16.0 ± 1.2	0.296 ± 0.010 A	10.4 ± 0.2	0.218 ± 0.014
	SWL	1.16 ± 0.08	15.2 ± 1.4	0.288 ± 0.010 AB	10.4 ± 0.2	0.230 ± 0.014
				$p = 0.041$		
Spinal column bone	WM	3,973 ± 60	46.9 ± 1.0	1.03 ± 0.04 B	129 ± 2 A	2.42 ± 0.04
	MWL	3,806 ± 90	44.5 ± 1.5	1.21 ± 0.04 A	119 ± 3 B	2.33 ± 0.06
	SWL	3,804 ± 101	46.6 ± 1.4	1.33 ± 0.08 A	119 ± 38 B	2.29 ± 0.04
				$p = 0.002$	$p = 0.04$	

Abbreviations: WM, weight maintenance; MWL, moderate weight loss; SWL, substantial weight loss. Data are means ± standard errors (n = 10–13). p-Values are from analysis of variance tests of group differences. Concentrations for each organ that are not marked with the same letter differ significantly (Duncan's test, $p < 0.05$).

Table 3. Organ contents of essential metals.

Organ	Group	Organ weight (g)	Calcium (μmol)	Copper (nmol)	Iron (μmol)	Magnesium (μmol)	Zinc (μmol)
Brain	WM	1.92 ± 0.03	3.12 ± 0.56	79.0 ± 1.8	0.60 ± 0.02	12.1 ± 0.26 A	0.36 ± 0.01
	MWL	1.88 ± 0.02	3.30 ± 0.88	75.2 ± 1.3	0.62 ± 0.01	11.7 ± 0.14 AB	0.36 ± 0.01
	SWL	1.83 ± 0.04	2.43 ± 0.31	75.7 ± 2.2	0.59 ± 0.02	11.2 ± 0.28 B	0.34 ± 0.01
						$p = 0.029$	
Femur	WM	0.63 ± 0.02	3,196 ± 46	32.6 ± 0.9	0.44 ± 0.01 B	101.3 ± 1.8	1.77 ± 0.02
	MWL	0.62 ± 0.02	3,056 ± 52	44.5 ± 10.1	0.55 ± 0.04 AB	95.6 ± 2.2	1.71 ± 0.04
	SWL	0.61 ± 0.01	3,027 ± 59	33.3 ± 0.6	0.60 ± 0.05 A	95.4 ± 2.2	1.69 ± 0.05
					$p = 0.036$		
Kidney	WM	0.79 ± 0.02 A	6.71 ± 2.36	196 ± 16 A	1.37 ± 0.06	6.95 ± 0.26 A	0.29 ± 0.01 A
	MWL	0.71 ± 0.02 B	9.24 ± 3.21	113 ± 5 B	1.33 ± 0.06	6.18 ± 0.20 B	0.26 ± 0.01 B
	SWL	0.60 ± 0.01 C	7.34 ± 2.13	113 ± 7 B	1.25 ± 0.04	5.02 ± 0.16 C	0.23 ± 0.01 C
		$p < 0.001$		$p < 0.001$		$p < 0.001$	$p < 0.001$
Liver	WM	9.38 ± 0.43 A	6.07 ± 0.33 A	607 ± 31 A	49.3 ± 2.2 A	76.3 ± 3.8 A	3.58 ± 0.17 A
	MWL	6.75 ± 0.17 B	4.53 ± 0.15 B	485 ± 10 B	43.4 ± 1.5 B	58.4 ± 1.4 B	3.02 ± 0.09 B
	SWL	5.61 ± 0.15 C	3.53 ± 0.16 C	412 ± 16 C	42.3 ± 1.3 B	47.1 ± 1.4 C	2.39 ± 0.08 C
		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.015$	$p < 0.001$	$p < 0.001$
Spinal column bone	WM	0.95 ± 0.03	3,739 ± 131 A	44.2 ± 1.8	0.96 ± 0.03 B	121 ± 5 A	2.28 ± 0.08
	MWL	0.94 ± 0.03	3,559 ± 89 AB	41.6 ± 2.1	1.13 ± 0.05 AB	111 ± 4 AB	2.18 ± 0.07
	SWL	0.88 ± 0.03	3,266 ± 140 B	40.8 ± 3.1	1.20 ± 0.08 A	102 ± 4 B	1.99 ± 0.08
			$p = 0.049$		$p = 0.038$	$p = 0.023$	

Abbreviations: WM, weight maintenance; MWL, moderate weight loss; SWL, substantial weight loss. Data are means ± standard errors (n = 10–13). p-Values are from analysis of variance tests of group differences. Contents for each organ that are not marked with the same letter differ significantly (Duncan's test, $p < 0.05$). Data for swimming and nonswimming rats are combined.

femur density was significantly reduced in nonswimming rats with weight loss induced by food restriction.

In this study, swimming rats consumed about 3 more grams of feed per day than the nonswimming rats during the 4-week period of food restriction in order to maintain body weights similar to those of their nonswimming counterparts. Although swimming did not influence the concentrations of blood Pb, organ Pb, and other essential elements in this study, it did help maintain femur density. This result is in agreement with other studies that demonstrate a beneficial effect of swimming on bone density in rats (*21*).

Blood Pb concentrations decreased gradually after the cessation of Pb exposure, as expected. However, during the food restriction period, the SWL rats consistently had the highest blood Pb concentrations for both swimming and, especially, nonswimming rats. The higher blood Pb concentrations of the SWL rats may be due to mobilization of Pb from the skeleton and noncalcified soft tissues that are reduced in mass by weight loss. Although bone Pb concentrations were not significantly reduced by weight loss, the substantial Pb stores in the skeleton could be the source of increased blood Pb concentrations because only relatively small decreases in bone Pb are required to substantially increase blood Pb. In addition, Pb in plasma at any time is determined almost entirely by the balance among excretion, and transfers to and from bone in the absence of new Pb exposure (*26,27*). The nonswimming SWL rats had higher blood concentrations than the swimming SWL rats. That may be related to the observation that the nonswimming rats lost more bone mass with weight loss; the results demonstrate that bone density was significantly reduced for the nonswimming but not the swimming rats during food restriction. Because most of the Pb in the circulation is bound to erythrocytes, another possibility is that blood Pb is raised due to the increased hematocrits of the food restricted rats. The significant correlations between hematocrit and blood Pb support this possibility. Thus, the mechanisms by which blood Pb concentrations increase subsequent to weight loss may include both increased concentrations of circulating erythrocytes and mobilization of Pb from bone.

The current study confirmed previous findings which demonstrated that liver and kidney Pb concentrations were significantly increased with weight loss induced by food restriction (*19*). The food restricted rats consumed 20–40% less Ca, Fe, and Mg than *ad libitum* controls; these nutrients, when ingested simultaneously with Pb, can interfere with and reduce gastrointestinal Pb absorption. However, Pb exposure occurred before the initiation of food restriction; thus, the reduced intakes of these three nutrients cannot explain the increased blood and organ Pb concentrations in the food restricted rats.

Organ Fe concentrations increased during weight loss for all organs except brain, even through daily Fe intake was reduced by 20–40% during food restriction. In contrast, organ concentrations of Ca, Cu, Mg, and Zn generally did not increase with weight loss (except for liver Cu and Zn), and the total content of these metals was decreased in several organs (Tables 2 and 3). Iron proteins are required for delivery of oxygen for tissue respiration. Furugouri (*28*) reported that Fe storage does not change significantly even during extended periods of fasting or starvation. It appears that mechanisms have evolved

to maintain Fe status during weight loss, perhaps because of the critical role of this micronutrient in respiration, and the same or similar mechanisms may act to conserve Pb.

The liver is the key organ for metabolizing exogenous toxicants, but its role in Pb detoxification is not clear. The liver contains numerous proteins to which Pb may bind. One of these proteins is metallothionein (*29*), which has a high affinity for Pb *in vitro* but not *in vivo* (*30*), although Pb can induce metallothionein production in liver (*31,32*). It is possible that the increased liver Pb during weight loss may include Pb bound to this protein, but more likely involves other proteins known to bind Pb *in vivo* (*33–35*). Fortunately, the liver is not sensitive to Pb toxicity in comparison to other organs such as the brain and kidneys, and the increase in liver Pb during weight loss may not result in overt toxic effects. On the other hand, diverting Pb to the liver may protect other organs more sensitive to Pb toxicity.

Bone contains minerals such as Ca, Mg, and trace elements and is constantly remolding, especially during weight loss. It functions as a reservoir to regulate mineral homeostasis under the influence of hormones, diet, exercise, and other factors. Bone is not just a storage site for Pb but also a target organ and an endogenous source of Pb exposure (*36,37*). Therefore, like Ca and Mg, Pb stored in bones may be released with bone remolding induced by various factors such as weight loss, pregnancy (*38–40*), menopause (*41*), bone diseases (*42*), and other pathophysiologic factors. The present study shows that femur density was significantly reduced with weight loss during food restriction in nonswimming rats. Using dual energy X-ray absorptiometry, other investigators have shown that bone density and strength decrease with bone mass loss during weight loss induced by food restriction or disease (*3–7*). Lead concentrations of bones did not show significant changes with weight loss or with non-weight-bearing exercise in this study. Because the bone Pb concentration is very high, a small portion released from bone can significantly increase soft tissue Pb concentrations, but atomic absorption spectrophotometry is not sensitive enough to demonstrate small percentage changes in the Pb concentrations and contents of bone.

In summary, this study demonstrates that food restriction and the accompanying weight loss result in increased blood, liver, and kidney Pb concentrations; higher hematocrits; and reduced bone density. Swimming during food restriction did not influence blood and organ Pb and essential metal concentrations but did prevent a decrease in femur bone density. Iron stores were conserved during weight loss, but Ca, Cu, Mg, and Zn were depleted in several organs. Thus, changes in Pb distribution during weight loss resemble those of Fe, but not the other divalent metals studied, and suggest that mechanisms involved in Pb and Fe conservation may be similar. If these results are applicable to humans, voluntary or involuntary weight loss will result in increases in blood and some organ Pb concentrations in people with prior excessive Pb exposure, and may produce toxic effects on those cells and organs sensitive to Pb toxicity.

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